CLAIMS

1. *In vitro* serological method for diagnosing microbial agents by immunodetection, wherein the presence is detected and, preferably, the quantity of patient immunoglobulins is assayed of both classes M and G, or only class G, specific to a microbial antigen characteristic of said microbial agent, in a patient's serum sample to be tested, by detection and preferably by quantification of an immunological reaction complex between said microbial antigen to be detected and a said specific, class M immunoglobulin for IgM assay and/or respectively a said specific, class G immunoglobulin for IgG assay using a first detection substance and/or respectively a second detection substance, preferably an antibody only reacting with a said immunoglobulin of the patient species of class M and/or respectively G, characterized in that:

1/ The steps are performed in which:

■ said serum sample to be tested is contacted with said first and second detection substances, or only said second detection substance, and with at least one solid support on which the following antigens have been attached:

- a first control antigen corresponding to a non-specific class G immunoglobulin of the patient species, and
- a second control antigen containing DNA/histone complexes, preferably all or part of nucleate cells comprising nuclei of nucleate cells, further preferably continuous line cells,
- optionally, a third control antigen corresponding to a non-specific class M immunoglobulin of the patient species, the presence of said third control antigen being necessary in the event of IgM assay, and
- at least one said microbial antigen, and

■ a series of controls is conducted comprising:

a- controlling the reactivity of said second detection substance by verifying whether said first control antigen reacts with said second detection substance, and optionally control of the presence of rheumatoid factors in said serum sample by verifying whether the first control antigen reacts with said serum sample and said first detection substance, in the event of IgM assay,

b- controlling the presence of anti-nuclear antibodies in said serum sample to be tested by verifying whether said second control antigen reacts with said serum sample and second detection substance,

c- controlling the reactivity of said first detection substance by verifying whether said third control antigen reacts with said first detection substance, in the event of IgM assay, and

d- controlling the presence of a human serum in the sample to be tested, and

2/ A reaction result between said microbial antigen, said serum sample and a said detection substance is only taken into account if the control of the presence of a human serum is positive and if the following accumulative conditions are met determining the absence of anti-nuclear antibodies and the reactivity of said second and, when applicable, said first detection substance, and optionally absence of rheumatoid factor:

a- said first control antigen reacts with said second detection substance,

b- said second control antigen does not react, and

c- when applicable, if said third control antigen reacts with said first detection substance, in the event of IgM assay.

2. Method as in claim 1, characterized in that a single solid support is used, contacted optionally simultaneously with said first and second detection substances containing a first and respectively a second labelling element, the second labelling element emitting a different signal to the first labelling element, preferably said first and second detection substances containing a first and respectively a second antibody only reacting with a said immunoglobulin of the patient species of class M and respectively of class G.

3. Method as in claim 1 or 2, characterized in that it is controlled that said tested sample does contain a serum of the patient species by detecting whether immunoglobulins of the patient species react with a fourth control antigen containing protein A of a *Staphylococcus aureus* bacterium, preferably said fourth antigen being a whole *Staphylococcus* bacterium, by contacting said sample with a solid support on which a said fourth control antigen is attached in the presence of said second detection substance which is a substance reacting with an immunoglobulin of the patient species and not reacting with said fourth control antigen, preferably an anti-immunoglobulin antibody of the patient species not reacting with said fourth control antigen, the control of the presence of a serum being positive if said fourth antigen reacts with said serum sample and said second detection substance.

4. Method as in any of claims 1 to 3, characterized in that said second detection substance is an animal immunoglobulin, preferably a goat or chicken immunoglobulin.

5. Method as in any of claims 1 to 4, characterized in that, optionally, the two said first and second detection substances are goat or chicken immunoglobulins respectively anti-IgM and anti-IgG.

6. Method as in any of claims 1 to 5, characterized in that said second control antigen consists of non-confluent cells in suspension, preferably human fibroblast cells.

7. Method as in any of claims 1 to 6, characterized in that said control antigens and microbial antigens are attached to the solid support by physical adsorption.

8. Method as in any of claims 1 to 7, characterized in that said microbial antigen is a corpuscular antigen consisting of a whole inactivated microbe or microbe fraction.

9. Serological diagnosis method as in any of claims 1 to 8, characterized in that said microbial agent is chosen from among the micro-organisms comprising a bacterium, a virus, a parasite or a fungus.

10. Serological diagnosis method as in claim 9, characterized in that said microbial antigen is an intracellular bacterium or a virus.

11. Serological diagnosis method as in claim 9 or 10, characterized in that said microbial antigen is chosen from among the bacteria of genus *Rickettsia, Coxiella, Bartonella, Tropheryma, Ehrlichia, Chlamydia, Mycoplasma, Treponema, Borrelia* and *Leptospira*.

12. Serological diagnosis method as in claim 11, characterized in that said microbial antigen is a bacterium responsible for endocarditis.

13. Serological diagnosis method as in claim 10, characterized in that said microbial antigen is a viral antigen chosen from among the viruses H.I.V, C.M.V., Epstein-Barr, Measles, Rubella, Hepatitis A and B.

14. Method as in any of claims 1 to 13, characterized in that:

- the detection, and preferably the assay is performed of the quantity of patient immunoglobulins of both classes M and G specific to a microbial antigen,
- at least one said microbial antigen and said first, second and third and fourth control antigens are attached to one same solid support, and
- for the detection of the different said microbial antigens the same said first and second detection substances are used with different labelling elements, said first and second detection substances being animal immunoglobulins not reacting with said fourth antigen, preferably in a human patient, chicken or goat immunoglobulins.

15. Method as in any of claims 1 to 14, characterized in that as solid support a glass or plastic slide is used, or a titre tube or well of a plastic microtitre plate.

16. Method as in any of claims 1 to 15, characterized in that in the sample to be tested the detection and, optionally the assay is performed of said immunoglobulin of the patient species specific to said microbial antigen, and the immunological reactions between said control antigens and said

detection substances are read by automated reading using appropriate reading equipment for said labelling elements, preferably equipment for reading a fluorescent signal of a fluorescent substance corresponding to the labelling elements of said detection substances.

17. Method as in any of claims 1 to 16, characterized in that said microbial antigen is a vaccine antigen and said immunoglobulin specific to said vaccine agent to be detected is a class G immunoglobulin.

18. Method as in claim 17, characterized in that the vaccine status of a person is determined by detection, preferably quantification of IgG serum antibodies specific to the vaccine antigens of a plurality of pathogenic agents of bacterial, viral, fungal or parasitic type, by detecting, and preferably quantifying, an immunological reaction complex between each said vaccine antigen and respectively each said antibody specific to said vaccine antigen, which may be present in a human serum sample to be tested, comprising:

1. Contacting one single, same said serum sample to be tested with:

- one same solid support on which a plurality of said vaccine antigens is attached corresponding to a plurality of pathogenic agents, and said first, second and preferably fourth control antigens,
- in the presence of at least one said second detection substance reacting with at least one said specific antibody and not reacting with any of said vaccine antigens, and

2. Performing at least one said control of the reactivity of said second detection substance using a said first control antigen, and one said control of

the presence of anti-nuclear antibodies using at least one said second control antigen, and controlling the presence of human serum in said sample to be tested preferably using a said fourth control antigen.

19. Method as in claim 18, characterized in that said specific antibodies of IgG immunoglobulin type are detected and a said second detection substance is used which is an antiIgG immunoglobulin, preferably a goat or chicken immunoglobulin.

20. Method as in either of claims 18 or 19, characterized in that said vaccine antigens are antigens of pathogenic agents chosen from among the viruses of mumps, rubella, measles, chicken pox, poliomyelitis, yellow fever, tick-borne encephalitis, hepatitis A, hepatitis B and the bacteria of *Bordetella pertussis*, tetanus and diphtheria.

21. Method as in any of claims 2 to 20, characterized in that it is determined whether the concentration of said specific antibodies reaches a threshold on and after which said specific antibody has a protective action protecting against the disease determined by the pathogen.

22. Method as in any of claims 18 to 21, characterized in that a determined volume of whole blood is collected using a capillary tube in a flask containing a determined volume of buffer allowing elution of the serum, the serum then preferably being diluted to a determined concentration, preferably 1:100 to 1:20.

23. Method as in any of claims 18 to 22, characterized in that, for each detection and optionally quantification of a said vaccine antigen, the following measurements are made:

1- a first measurement of a first value representing the quantity of a first labelling element, preferably the first intensity value of a signal emitted by said first labelling element, further preferably fluorescent, said first labelling element binding itself non specifically to any protein in the depositing area of said vaccine antigen, and

2- a second measurement of a second value representing the quantity of a second labelling element emitting a different signal to said first labelling element, preferably a second value of the intensity of the signal emitted by this second labelling element, also preferably fluorescent at a different excitation wavelength to that of the first fluorescent labelling element, said second labelling element being the labelling element of said second detection substance for said vaccine antigen in the depositing area of said antigen, and

3- the ratio between said first and second values is calculated, and

4 - the value of said ratio is compared with the value of a reference ratio obtained with a collection of positive and negative reference sera, thereby making it possible to determine, by comparison, whether or not it is necessary to vaccinate the person against said vaccine antigen in relation to the ratio between said first and second values.

24. Diagnosis kit which can be used for implementing a method as in any of claims 1 to 23, characterized in that it contains:

- at least one said solid support, preferably a single solid support, on which at least one said microbial antigen and said control antigen(s) are attached, and

- said detection substance(s) and reagents used to develop said labelling element(s).

25. Kit as in claim 24, characterized in that it contains:

- one same said solid support on which at least one said corpuscular antigen and said control antigens are attached by physical adsorption, and
- at least one of a same said first or second detection substance to detect the different microbial antigens.

26. Kit as in claim 24 or 25, characterized in that it comprises a flask containing a determined volume of elution buffer to collect a determined volume of serum sample to be tested.

27. Method for preparing a solid support on which at least one antigen is attached chosen from among a said microbial agent, preferably corpuscular, a said first, a said second, optionally a said third and preferably a said fourth control antigen enabling detection by automated reading using a said first and optionally said second detection substance, which can be used in a method as in any of claims 1 to 23, or a kit as in any of claims 24 to 26, characterized in that said microbial antigens, preferably corpuscular, and control antigens are deposited by robot arrayer which preferably comprises a syringe, said corpuscular antigens preferably being associated with a dye, preferably a fluorescent dye in the form of a suspension at a concentration enabling their visualization with said dye after being deposited, thereby making it possible to verify the attachment of said antigens to said solid support.

28. Method as in claim 27, characterized in that a robot arrayer is used to deposit a said microbial antigen and optionally a said second control antigen and optionally a said fourth control antigen in the form of a suspension of non-confluent cell corpuscles, whole viruses or whole bacteria, or fractions of cells or bacteria.

29. Method as in claim 28, characterized in that the control antigens in the form of a cell suspension are calibrated at a concentration of 10^7 to 10^9 cells/ml, said control antigens or microbial antigens in the form of suspensions of bacteria or bacteria fractions are calibrated at a concentration of 10^7 to 10^9 particles/ml, and the suspensions of whole viruses at a concentration of 10^9 to 10^{10} particles/ml.

30. Method as in either of claims 28 or 29, characterized in that said control antigens and corpuscular microbial antigens are deposited in a mixture with a protein binder to stabilize attachment to said solid support.

31. Method as in claim 30, characterized in that said protein binder is chosen from among egg yolk, gelatine, bovine serum albumin or a non-human polyclonal IgG, preferably goat.

32. Method as in claim 31, characterized in that said corpuscular microbial antigen is deposited on said solid support consisting of a glass slide, in a mixture with an immunoglobulin of goat polyclonal IgG type.

33. Method as in any of claims 27 to 32, characterized in that prior washing of said solid support is performed with

a solution of an ethanol/acetone mixture, preferably 50-50, then the said antigens are deposited and their attachment stabilized by physical adsorption on said solid support by treatment with alcohol, preferably methanol or ethanol, which alcohol is subsequently removed, further preferably the attachment of said antigens is verified by staining, preferably with fluorescent labelling non-specific to the proteins or DNA.

34. Method as in any of claims 27 to 32, characterized in that the attachment by physical adsorption of said control antigens and microbial antigens is completed by cross-linking treatment, preferably a chemical treatment using a bi-functional agent for covalent coupling.